

Exhibit

To  
Ralph M. STEINMAN, M.D.  
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Dear Ralph,

I would like to give you a brief update on a few things.

As you

know we have been successful to culture DC as proliferating balls from cord blood as well as blood and bone marrow from patients that had been treated with G-CSF or GM-CSF. For that we had cultured the MHC class II and CD3 negative fractions of PBMC obtained by panning in GM-CSF +/- several other cytokines, and got "our" proliferating DC balls as observed in the mouse. When we cultured such fractions which we had obtained from PBMC of normal, healthy individuals we got - as I had already told you - proliferating balls within a few days. These balls looked like developing DC balls in overall appearance including some initial veins at the edges, yet the balls did not develop further and even started to die. When we learned from Una that freshly isolated DC need monocyte conditioned medium for staying viable we wondered whether we had depleted monocytes via their Fc receptor as a side effect of our panning procedure. One should not forget that for the latter we use whole anti-murine Ig antibody for coating petridishes and pan the cells at room temperature at least for a while. Although we planned and still plan to test panning using a  $F(ab')_2$  anti-murine Ig for panning (as well as using magnetic beads as an alternative) I thought it might be useful while waiting for the ordered antibody to try the simple protocol I had used for mouse blood. So we plated exactly as described in the mouse PBMC at  $1 \times 10^6$  / ml / 24 well + GM-CSF or GM-CSF plus TNF alpha, and transferred the NAF after 1 day. As in the mouse (one

should note that I myself always got the balls mainly in the adherent fraction in contrast to Kayo who presumably washed more vigorously) we got nice proliferating DC balls although already on day 3 ! Then we repeatedly observed - with GM-CSF more rapidly than with GM-CSF plus TNF alpha - that the balls switched and turned into what we have been calling "bad balls" in our numerous experiments with cord blood and GM-CSF / G-CSF blood. Would'nt there be my experience from all these experiments and would'nt I have looked at the cultures twice a day on 7 days a week we for sure had missed that the switch occurs virtually overnight. Basically we had observed this switching phenomenon though not so impressively in our experiments with blood from G-CSF or GM-CSF treated patients and rarely even with cord blood. Taking off the non-adherent fractions retards the phenomenon also with normal blood. As IL-4 has been described to inhibit macrophage colony formation [ Jansen, J.H. et al. Inhibition of human macrophage colony formation by Interleukin 4, J. Exp. Med. 170:577-582 (1989)] we tried to add IL-4 to prevent macrophage development or switching to "bad balls". Remarkably this was indeed the case, and even more surprisingly the DC balls were not affected or inhibited. So IL-4 is clearly not essential for DC development yet is useful via blocking macrophage development. We are now trying to perform basic functional tests and phenotyping.

Given these recent developments I would be grateful when you could let me know when I could reach you by phone as I would like to discuss how we should now proceed in the human DC project. As planned for 2 months (at least) I will try to write the DC culture stuff together.

Sincerely Yours,

Gerold SCHULER, M.D.

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University Hospital

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A handwritten signature in black ink, appearing to read "Gerold Schuler". The signature is fluid and cursive, with a stylized "G" at the beginning.